Kidney International, Vol. 46 (1994), pp. 1422–1430

Appearance of tumor necrosis factor-α and soluble TNF-receptors I and II in peritoneal effluent of CAPD

DÉSIRÉE ZEMEL, ALEXANDER L.T. IMHOLZ, DIRK R. DE WAART, CHRIS DINKLA, DIRK G. STRUIJK, and RAYMOND T. KREDIET

Renal Unit, and Laboratory of Clinical Chemistry, Academic Medical Center, Amsterdam; and Foundation for Home-Dialysis Midden-West Nederland, Utrecht, The Netherlands

Appearance of tumor necrosis factor-α and soluble TNF-receptors I and II in peritoneal effluent of CAPD. Dialysate and serum concentrations of tumor necrosis factor-α (TNF-α), soluble TNF-receptor I (sTNFRI) and soluble TNF-receptor II (sTNFRII) were measured during stable and infectious CAPD to determine whether these mediators are released intraperitoneally or derived from the circulation. Dialysate/serum ratios were compared to those of various marker proteins for peritoneal transport and to interleukin-6 (IL-6), which is locally produced. Peritoneal immunoreactive TNF-α could be detected in 19 of 20 stable CAPD patients after a night dwell, but only occasionally and in lower concentrations during and after a standard four-hour peritoneal permeability test. Both sTNFRs highly exceeded TNF-α dialysate concentrations. In case of peritonitis a median 16-fold increase in dialysate TNF-α occurred on the first day, which declined towards control values during a longitudinal follow-up of eight consecutive days. sTNFRI and sTNFRII in dialysate increased three- to fourfold. Their peaks, however, appeared on the second peritonitis day. Bioactive TNF-α was only detected when immunoreactive levels exceeded 1000 pg/ml. Serum values of all variables were not altered during infection; sTNFRs exceeded TNF-α 300- to 400-fold. During stable CAPD indirect evidence was obtained for transperitoneal transport from plasma to dialysate of TNF-α (molecular wt 17 kD), sTNFRI (55 kD) and sTNFRII (75 kD). Dialysate/serum (D/S) ratios were higher, the lower the molecular weight; they were related to D/S ratios of those marker proteins with the nearest molecular weight; D/S ratios were unrelated to the intraperitoneally produced IL-6. Furthermore, the observed D/S ratios were as expected theoretically for their molecular weights. Higher than expected D/S ratios were found during peritonitis for TNF-α on days 1 and 2, and for sTNFRII on day 2, pointing to local release within the peritoneal cavity only in the acute inflammatory phase. Gel permeation chromatography revealed that TNF-α was present in a monomeric 17 kD form, unbound to receptors, whereas in case of peritonitis smaller sTNFRII fragments, transported at a higher rate, could not be excluded. Therefore, these higher than expected D/S ratios indicate local production of TNF-α during peritonitis and possibly also of sTNFRII, although transport of smaller receptor fragments might also occur.

Inflammatory mediators have attracted much attention as natural constituents of host defense mechanisms over the last few years [1–4]. Involvement of mediators in the context of peritoneal dialysis is also reflected by the presence of these substances in the peritoneal effluent. Some mediators are not merely transperitoneally transported from the circulation, but are produced locally within the peritoneal cavity, even in the absence of overt clinical infection. This has been demonstrated for the cytokines interleukin-6 (IL-6) and IL-8, and for eicosanoids [5–10]. However, preliminary reports on the presence and origin of the 17 kD cytokine tumor necrosis factor-α (TNF-α) have been concise and conflicting [11, 12]. TNF-α is a central mediator involved in the regulation of many biological responses. Apart from physiological properties TNF-α might have deleterious effects when produced excessively, since TNF-α is capable of inducing angiogenesis [13] and fibroblast proliferation [14]. Therefore, intraperitoneal production of this potentially harmful cytokine could theoretically lead to structural changes of the peritoneal membrane and might consequently affect the process of peritoneal dialysis.

Recently, two soluble TNF-receptors (sTNFRs) have been described that are able to bind free TNF-α: sTNFRI (molecular wt 55 kD) and sTNFRII (molecular wt 75 kD) [15]. These receptors represent the extracellular domains of two TNF-receptors that can be released from the cell surface by proteolytic cleavage [16]. Binding to these shedded soluble receptors might inactivate bioactive TNF-α and simultaneously reduce the sensitivity of cells to TNF-α mediated effects. Alternatively, the formation of such complexes might lead to a reservoir from which bioactive TNF-α can be released [17]. Increased plasma concentrations of sTNFRs have been described in patients with end-stage renal disease either before commencing renal replacement therapy or during hemodialysis [18–21]. During peritoneal dialysis serum proteins are transported from the circulation across the peritoneal membrane to the peritoneal cavity. Based upon their molecular weights, transperitoneal transport of sTNFRs would be expected to occur at a rate similar to albumin (69 kD) and IgG (150 kD). However, it is unknown whether these soluble receptors are in fact present in peritoneal effluent and if so, whether they appear in dialysate by peritoneal transport or are released locally within the peritoneal cavity.

The present study was aimed first to establish the concentrations of immunoreactive TNF-α, sTNFRI and sTNFRII in dialysate and serum of patients treated with CAPD during stable and infectious circumstances. This was accomplished in stable CAPD patients both during a night dwell and during a standardized four-hour peritoneal permeability test. The effect of inflammation was established in a longitudinal study performed on eight consecutive days from the start of peritonitis and once after

Received for publication March 21, 1994
and in revised form June 8, 1994
Accepted for publication June 9, 1994

Table 1. Patient characteristics of the studied patient groups

Group	1	2	3
N	20	7	12
M:F	10:10	5:2	8:4
Age *years*	56 (25–78)	55 (25–74)	49 (23–69)
CAPD duration *months*	25 (3–98)	3 (2–35)	17 (4–107)
Peritonitis incidence *ep/yr*	0.5 (0–5.1)	1.4 (0–4)	2.1 (0–3.9)
Renal disease:			
Diabetic nephropathy	7	—	5
Chronic glomerulonephritis	3	2	2
Chronic interstitial nephritis	1	—	2
Hypertensive nephropathy	3	3	1
Adult polycystic kidney disease	1	—	1
Others and unknown	5	2	1

Median values and ranges are given.

recovery. Bioactivity of peritoneal TNF-α was additionally studied in stable and infectious CAPD patients. The second objective was to investigate whether the presence of TNF-α and its sTNFRs in dialysate merely reflects transperitoneal transport from the circulation or local intraperitoneal release. Therefore, dialysate/serum ratios of these substances were compared to dialysate/serum ratios of marker proteins for peritoneal transport and to dialysate/serum ratios of the intraperitoneally-produced 26 kD cytokine, IL-6. These comparisons were made both in stable patients and in patients with peritonitis. Finally, gel permeation chromatography was performed on dialysate samples to assess the actual *in vivo* molecular weights of TNF-α, sTNFRI and sTNFRII during peritoneal dialysis.

Methods

Patients

In group 1 paired samples from night bags and serum (drawn the next morning) were obtained from 20 clinically stable CAPD patients. Patient characteristics are summarized in Table 1. Ten patients used 2 liter exchanges, 10 used 1.5 liter. Glucose 3.86% was employed by three patients, 2.27% by 5 and 1.36% by 12. Dwell times ranged from 7.3 to 11.5 hours (median 10).

In a separate group of seven stable CAPD patients (group 2, Table 1) TNF-α kinetics were studied during a standard four-hour peritoneal permeability test with glucose 1.36% as described by us previously [22]. Briefly, the peritoneal cavity was rinsed twice before each study. Dialysate samples were taken 10 minutes after instillation of the test bag, after one hour and after four hours. Blood samples were drawn prior to each test. Four patients used 1.5 liter and three patients used 2 liter exchanges. All patients of groups 1 and 2 were free of peritonitis at least three weeks prior to the time of the study.

In group three 12 CAPD patients were studied during 15 episodes of peritonitis (Table 1). Nine patients used 2 liter exchanges, and 3 used 1.5 liter exchanges. All were free to choose their appropriate glucose concentration. Patients were followed on eight consecutive days from the start of peritonitis and once after recovery from the infection (control). Peritonitis was diagnosed when two of the following three criteria were present: (1) dialysate leukocyte count exceeding 100×10^6/liter; (2) positive dialysate culture; (3) abdominal complaints [23]. After delivery of the first peritonitis bag to the hospital all subsequent night bags were collected. These were weighed, samples were drawn and stored immediately at −20°C. Serum samples were taken on day 1 and on the control day. All patients were treated initially with oral and intraperitoneal cephradine during the first 24 hours as described previously [24]. Briefly, patients received 125 mg/liter intraperitoneally (loading dose 250 mg/liter) and 250 mg orally at each dialysate exchange. Cephradine was continued orally after the first 24 hours or replaced by another antibiotic if indicated by the culture (8 cases). Antibiotics were continued for one week after the dialysate leukocytes had dropped below 100×10^6/liter and the culture had become negative. The control investigation was performed 14 days after this time point. All patients responded well to the appropriate antibiotic treatment and CAPD could be continued during and after peritonitis in all of them.

CAPD was performed in all patients with commercially available dialysate (Dianeal®, Baxter BV, Utrecht, The Netherlands). Informed consent was obtained from every patient after an explanation of the purpose and methods of the study. The protocol was approved by the committee of medical ethics of the University Hospital of Amsterdam.

Measurements

All samples were kept frozen at −20°C until analysis. Cephradine did not interfere with any of the performed determinations.

Immunoreactive TNF-α was measured in dialysate and serum of all groups with a commercially available ELISA (Medgenix, Brussels, Belgium) applying the instructions of the manufacturer. This assay employs recombinant TNF-α with a molecular weight of 17 kD. Addition of either recombinant soluble TNF-receptor I or soluble TNF-receptor II in concentrations exceeding TNF-α up to 10-fold did not have any effect on the measurement of TNF-α. This indicates that the employed ELISA detects both free TNF-α and TNF-α complexed with soluble TNF receptors. The lower detection limit after dilution of the standard was 2 pg/ml. Intra-assay variability was 7%, inter-assay variability 12%. Recovery of exogenous TNF-α from peritoneal fluid was 108 ± 14% (mean ± SEM).

Bioactive TNF-α was determined in peritoneal effluents of groups 1 (10 cases) and 3 ($N = 6$) using the murine fibrosarcoma WEHI 164 cell line [25]. The detection limit was 0.1 U/ml, corresponding with 1.6 pg/ml.

Soluble TNF-receptors were measured with a commercially available ELISA (R&D Systems, Minneapolis, Minnesota, USA) as recommended by the manufacturer. Briefly, 96-well polystyrene microtiter plates, coated with a murine monoclonal antibody against either sTNFRI or sTNFRII, were incubated with standards or diluted samples (using a mixture of diluents RD5 and RD6K) for two hours at room temperature. This was followed by three washing steps. Subsequently a polyclonal antibody against sTNFRI or sTNFRII was added and incubated at room temperature for two hours. After washing according to the instructions substrate solution containing hydrogen peroxide was added and incubated for 20 minutes. The color reaction was stopped with

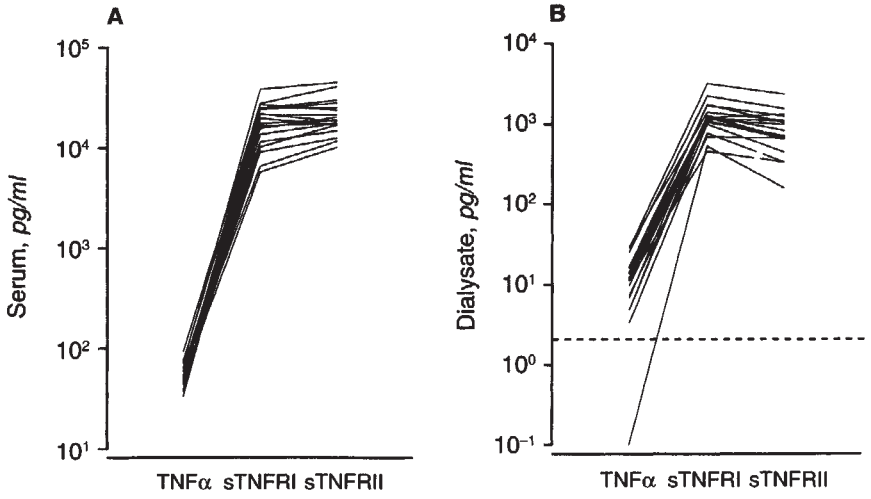

Fig. 1. *Serum (A) and dialysate (B) concentrations of immunoreactive TNF-α, sTNFRI and sTNFRII shown for each of the 20 stable CAPD patients studied after an overnight dwell (group 1).* The broken line indicates the detection limit for TNF-α. TNF-α vs. sTNFRI or sTNFRII, $P \leq 0.0001$, both in dialysate and in serum. sTNFRI vs. sTNFRII, $P < 0.0001$ in dialysate, $P = 0.006$ in serum.

sulfuric acid and the resulting color intensity was determined spectrophotometrically at a wavelength of 450 nm (EASIA reader, Medgenix, Brussels, Belgium). Both assays for sTNFRs do not cross-react with each other or with IL-1α, IL-1β, IL-2, IL-6, IFN-γ or TNF-α. The assays measure both free receptor and receptor bound to TNF-α. The detection limit was 7.8 pg/ml for both assays. Intra-assay variabilities were 4% for sTNFRI and 3% for sTNFRII. Inter-assay variability was 8% for sTNFRI and 7% for sTNFRII. Recoveries of exogenously added sTNFRs from peritoneal dialysate were always above 70%.

IL-6 was measured in dialysate and serum of groups 1 and 3 using an ELISA as described by Helle et al [26] with only minor modifications as previously reported by us [8]. The lower detection limit was 3 pg/ml. Intra-assay variability was 9%, inter-assay variability was 14%.

Albumin, IgG and α_2-macroglobulin were determined by immunonephelometry, using commercial antiserum (Dakopatts, Glostrupp, Denmark). β_2-microglobulin was measured by EIA (IMX, Abbott Diagnostics, Chicago, Illinois, USA). Coefficients of variation for the determinations were 5% for β_2-microglobulin and 3% for albumin, IgG and α_2-macroglobulin.

Gel permeation chromatography was performed on dialysate and serum samples of both stable and infected CAPD patients (days 1 and 2 of peritonitis) using a Superose 12 column with a total volume of 25 ml (Pharmacia, Uppsala, Sweden). Samples obtained during stable CAPD were concentrated tenfold. Gel permeation was done at 0.4 ml/min with sampling every minute. Fractions of 0.4 ml were collected (pH 7.4). Phosphate buffered saline served as eluent. Fractions were assayed for TNF-α and sTNFRs as described above. Thyroglobulin, aldolase, chymotrypsin, ribonuclease and cytochrome C standards (all from Pharmacia) were used to calibrate the column, as well as recombinant TNF-α (molecular wt 17 kD, Medgenix, Brussels, Belgium) and the dialysate concentrations of IgG and albumin. The exponential relationship between molecular weight and retention time yielded the following equation: log molecular wt = 7.751 − 0.094 × retention time. In addition serum samples from septic patients with or without acute renal failure served as controls.

Calculations

Dialysate/serum ratios (D/S) were calculated for TNF-α and sTNFRs (groups 1 and 3). These were compared to D/S ratios of both marker proteins for peritoneal transport and the intraperitoneally produced 26 kD cytokine IL-6. Expected D/S ratios of TNF-α and sTNFRs were determined assuming transperitoneal transport from serum to dialysate. This was done by interpolation, using the power relationship that exists between clearances or D/S ratios of serum proteins and their molecular weights [27]. In these calculations the molecular weights used in the stable patients group and on the control day of group 3 were 17 kD for TNF-α, 55 kD for sTNFRI and 75 kD for sTNFRII.

Statistics

Non-parametric tests were applied (Wilcoxon rank sum and Spearman-rank correlation test). The differences between observed and predicted D/S ratios of TNF-α and sTNFRs were tested by a modified *t*-test. This test takes the variability of the regression line between molecular weights and D/S ratios of marker proteins into account.

Results

TNF-α and soluble TNF receptor concentrations

Stable CAPD. Dialysate and serum concentrations of immunoreactive TNF-α, sTNFRI and sTNFRII in 20 clinically stable CAPD patients are shown in Figure 1 (group 1). Peritoneal TNF-α remained below the detection limit after an overnight dwell only in one patient. In dialysate, TNF-α concentrations were related with both sTNFRI and sTNFRII ($r \geq 0.71$, $P \leq 0.003$). In serum TNF-α was only related to sTNFRII ($r = 0.50$, $P = 0.03$). Dialysate TNF-α appeared biologically inactive in each patient (< 0.1 U/ml). TNF-α could not yet be detected in the dialysate of any patient of group 2 in the samples obtained 10 minutes after the start of a dwell, only in one patient after one hour (4 pg/ml) and in three of the patients after four hours (2, 4 and 5 pg/ml). In three patients studied after both a short and a long dwell, peritoneal TNF-α concentrations were higher in the night effluents than in the four hour dwells. When group 1 and 2 were compared TNF-α concentrations in dialysate were also significantly higher after an overnight dwell ($P < 0.001$).

Peritonitis. Most episodes were caused by gram-positive microorganisms: *Staphylococcus aureus* (6 cases), *Staphylococcus epidermidis* (3), *Streptococcus sanguis* (2), *Streptococcus mitis* (1) and

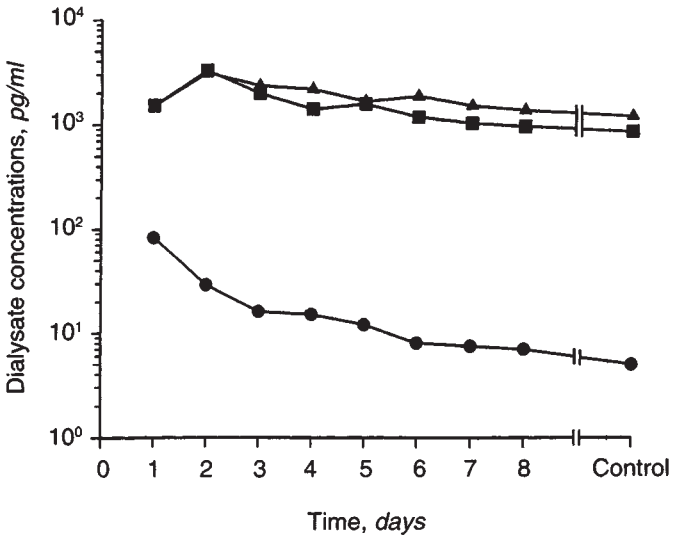

Fig. 2. *Median dialysate concentrations of TNF-α, sTNFRI and sTNFRII in 15 episodes of peritonitis (group 3).* Patients were followed on 8 consecutive days from the start of the infection and once after recovery (control, median day 22, range 19 to 35). Symbols are: (●) TNF-α; (▲) sTNFRI; (■) sTNFRII.

Streptococcus agalactiae (1). One infection was due to a gram-negative bacterium (Acinetobacter). One culture remained negative, even after prolonged culture for seven days. Figure 2 shows median dialysate concentrations of TNF-α, sTNFRI and sTNFRII during the acute phase and the recovery from peritoneal infection. TNF-α decreased from day 1 (median 82 pg/ml, range 12 to 1873 pg/ml) to the control day (5 pg/ml, range 2 to 23 pg/ml). In contrast, peak dialysate concentrations of both sTNFRs were observed on the second day ($P \leq 0.01$ vs. TNF-α). Median sTNFRI declined from 3113 pg/ml (range 1617 to 8367 pg/ml) on day 2 to 1196 pg/ml (526 to 2460 pg/ml) after recovery ($P < 0.001$); sTNFRII decreased from 3270 pg/ml (1307 to 23225 pg/ml) to 854 pg/ml (257 to 2232 pg/ml) ($P < 0.001$). Bioactivity of peritoneal TNF-α was determined on days 1 and 2 of peritonitis. Only in two samples with immunoreactive TNF-α levels exceeding 1000 pg/ml was low bioactivity detected, that could be blocked by anti-TNF-α. Serum concentrations measured during the acute inflammatory phase were neither different from those measured after recovery, nor from those observed in the stable patients of group 1 (Table 2). All three mediators were however higher than values reported for healthy individuals by the manufacturers of the applied assays. No correlation was present between dialysate TNF-α and sTNFRs on the first peritonitis day. On the control day peritoneal TNF-α was related to sTNFRII ($r = 0.73$, $P = 0.006$), not to sTNFRI ($r = 0.42$, $P = 0.12$). Similar to the stable patients of group 1, serum TNF-α after recovery was only related to sTNFRII ($r = 0.61$, $P = 0.02$).

Transperitoneal transport versus local production

Stable CAPD. Figure 3 shows D/S ratios of TNF-α and sTNFRs for each individual of group 1. All D/S ratios were lower than 1.0. Also, the D/S ratios were higher the lower the molecular weight, suggesting size-selective peritoneal transport rather than local synthesis. Table 3 summarizes these data in relation to D/S ratios of marker proteins for peritoneal transport and to D/S ratios of

Table 2. Serum concentrations (pg/ml) of TNF-α, sTNFRI and sTNFRII

		Peritonitis	
	Stable CAPD	Day 1	After recovery
TNF-α	64 (33–59)	59 (37–84)	53 (33–93)
sTNFRI	22200 (10316–32720)	17986 (9948–34220)	18041 (5786–38520)
sTNFRII	25020 (12860–38540)	23800 (14320–37980)	19020 (10160–45150)

Median values and ranges are given during stable CAPD ($N = 20$, group 1) and during the acute phase and recovery from peritonitis ($N = 15$, group 3).

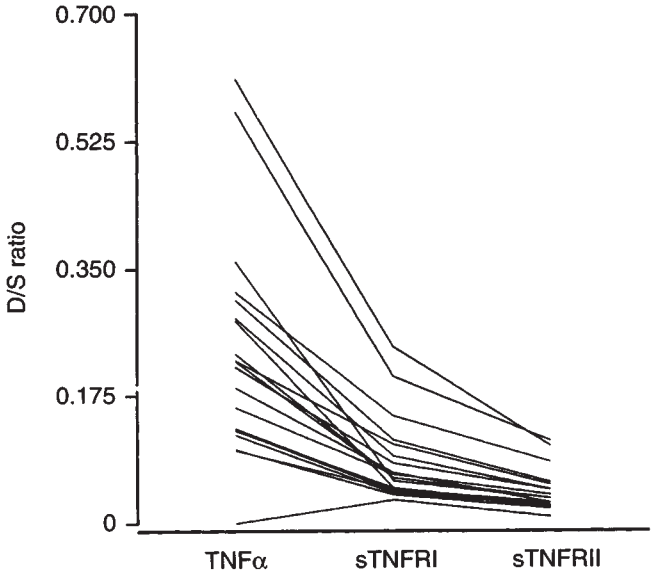

Fig. 3. *Dialysate/serum (D/S) ratios for TNF-α (17 kD), sTNFRI (55 kD) and sTNFRII (75 kD) during stable CAPD (group 1).* $P \leq 0.0002$ for all differences between all variables.

the intraperitoneally produced IL-6. In contrast to IL-6 (26 kD), D/S ratios of TNF-α and sTNFRs were in the range as expected for transport of solutes with these molecular weights. Rank correlations were found for $D/S_{TNF-\alpha}$, D/S_{sTNFRI} and $D/S_{sTNFRII}$ with D/S ratios of marker proteins. The best correlations are shown in Figure 4. In contrast, $D/S_{TNF-\alpha}$, D/S_{sTNFRI} or $D/S_{sTNFRII}$ were not related to D/S_{IL-6} (Fig. 4). D/S_{IL-6} appeared also unrelated to any of the marker proteins. $D/S_{TNF-\alpha}$ correlated with D/S ratios of both sTNFRs ($r \geq 0.83$, $P \leq 0.002$); D/S_{sTNFRI} was related to $D/S_{sTNFRII}$ ($r = 0.97$, $P < 0.001$).

The linear relationship that exists between D/S ratios of marker proteins for peritoneal transport and their molecular weights when plotted on a double logarithmic scale is given in Figure 5. During stable dialysis the observed $D/S_{sTNFRII}$ was similar to the predicted value for transport of a globular protein with a molecular weight of 75 kD (upper left panel). For TNF-α and sTNFRI the observed D/S ratios seemed to be higher than expected, since they appeared above the line for transperitoneal transport. However, the difference was not statistically significant. Thus, both TNF-α and sTNFRs are probably transported from the circulation

Table 3. D/S ratios of TNF-α and sTNFRs in relation to D/S ratios of marker proteins for peritoneal transport and the locally produced cytokine IL-6

	Molecular wt	D/S ratio			
			Peritonitis		
		Stable CAPD	Day 1	Day 2	After recovery
β_2-microglobulin	11.8	0.264 (0.128–0.588)	0.224 (0.111–0.727)	0.354 (0.236–0.612)	0.245 (0.138–0.423)
TNF-α	17	0.218 (0–0.610)	1.455 (0.233–27.544)	0.413 (0.107–4.791)	0.100 (0.033–0.277)
sTNFRI	55	0.062 (0.032–0.242)	0.084 (0.031–0.222)	0.157 (0.078–0.370)	0.066 (0.028–0.110)
albumin	69	0.024 (0.014–0.070)	0.032 (0.011–0.101)	0.054 (0.032–0.075)	0.029 (0.013–0.047)
sTNFRII	75	0.034 (0.009–0.113)	0.051 (0.012–0.195)	0.117 (0.051–1.749)	0.036 (0.007–0.072)
IgG	150	0.015 (0.006–0.042)	0.022 (0.008–0.059)	0.029 (0.016–0.038)	0.016 (0.007–0.026)
α_2-macroglobulin	820	0.003 (0.001–0.030)	0.009 (0.001–0.028)	0.012 (0.004–0.030)	0.004 (0.001–0.010)
IL-6	26	26 (0–57)	2519 (2–15500)	526 (48–12473)	9 (0.5–65)

Median values and ranges are given during stable CAPD (N = 20, group 1) and during the acute phase of and after recovery from peritonitis (N = 15, group 3).

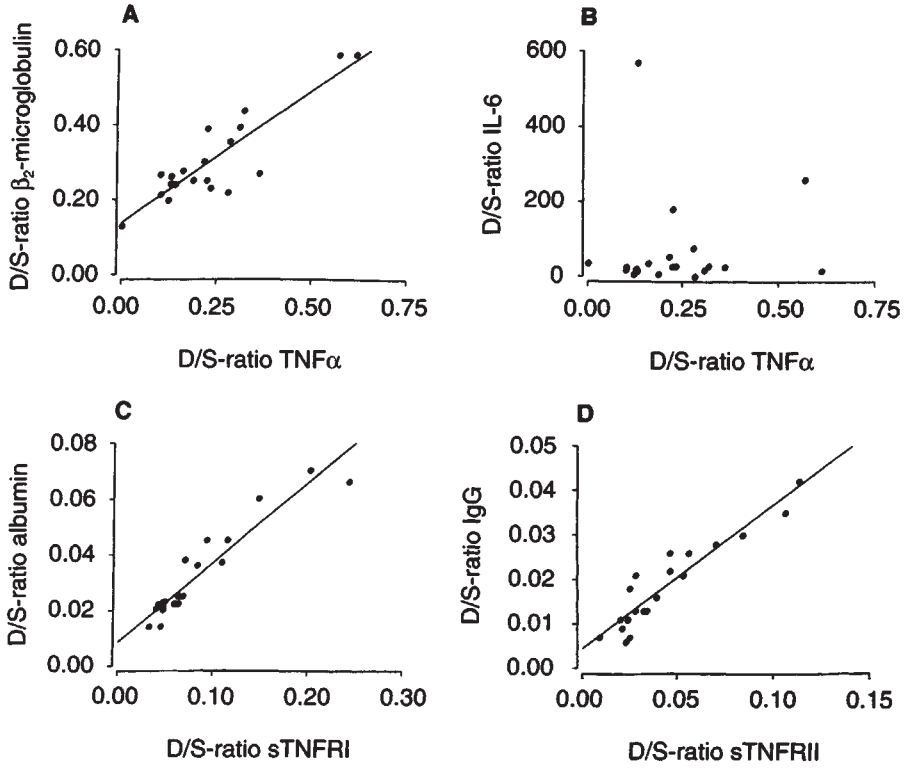

Fig. 4. *Spearman rank correlations between D/S ratios of TNF-α (17 kD), sTNFRI (55 kD) and sTNFRII (75 kD) with marker proteins for peritoneal transport with a similar molecular weight.* No correlation was present with the intraperitoneally produced 26 kD cytokine IL-6 (shown for TNF-α in **B**). **A.** P = 0.002, r = 0.721. **B.** r = 0.078, NS. **C.** P = 0.001, r = 0.924. **D.** P = 0.0001, r = 0.908.

across the peritoneal membrane to dialysate during stable dialysis, similar to marker proteins for peritoneal transport.

Peritonitis. Table 3 summarizes the D/S ratios of TNF-α, sTNFRI and sTNFRII during the first two days of peritonitis and after recovery (group 3). The observed D/S ratios during these days are plotted relative to the expected values based upon transperitoneal transport in Figure 5. The observed $D/S_{TNF\text{-}\alpha}$ was significantly higher than predicted on the first two days of the infection ($P < 0.001$, Fig. 5, upper and lower right panels). On the second day of peritonitis the observed D/S ratio of sTNFRII was also significantly higher than expected for transport of a 75 kD solute ($P < 0.02$). The lower left panel shows that all D/S ratios were within the expected range after recovery from peritonitis, similar to the stable patients of group 1.

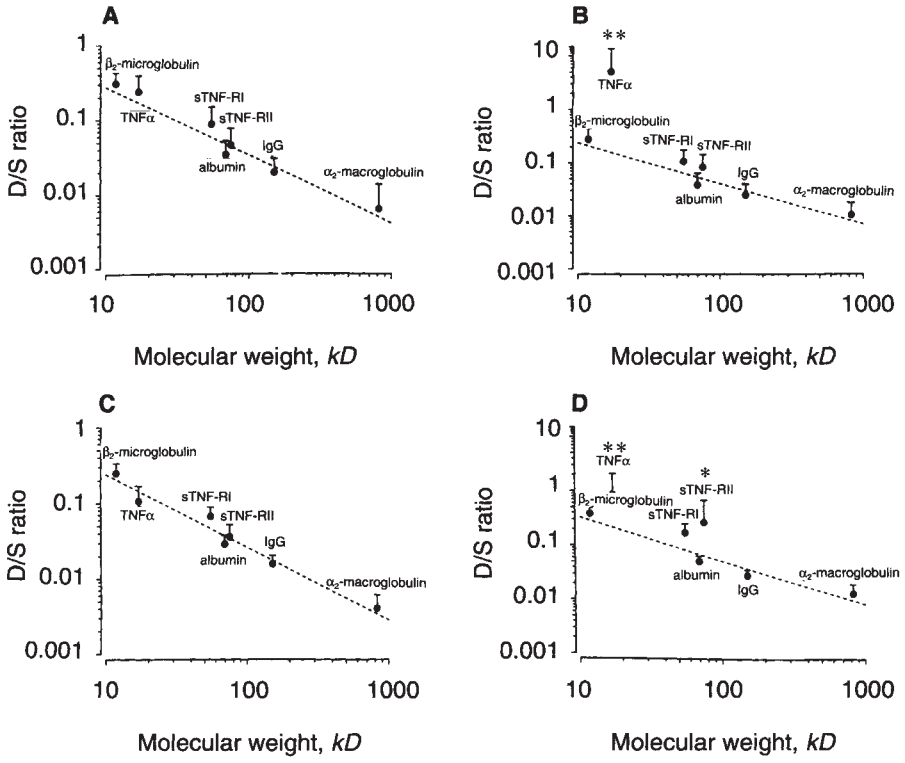

Fig. 5. *Regression lines based upon the relationship between D/S ratios of marker proteins for peritoneal transport with their molecular weights.* Positions of TNF-α, sTNFRI and sTNFRII relative to this transport line are given during various conditions. **A.** stable CAPD (N = 20, group 1). **B.** peritonitis day 1 (N= 15, group 3). **C.** after recovery from peritonitis (N = 15, group 3). **D.** peritonitis day 2 (N = 15, group 3). Mean values and 1 standard deviation are shown. * $P < 0.02$ for difference between the observed and the expected $D/S_{sTNFRII}$ for transport of a solute with a molecular weight of 75 kD; ** $P < 0.001$ for difference between the observed and the expected $D/S_{TNF\text{-}\alpha}$ for transport of a solute with a molecular weight of 17 kD.

Gel permeation chromatography. To examine whether molecular sizes of TNF-α and sTNFRs were different during peritoneal infection, dialysate samples obtained during peritonitis were analyzed by gel permeation chromatography and compared to concentrated dialysate derived from stable CAPD patients (Fig. 6). The TNF-α peak in dialysate eluted in the same fraction as recombinant monomeric TNF-α (17 kD) or TNF-α in serum from septic patients, irrespective whether the dialysate was obtained during peritonitis or stable CAPD. TNF-α did not elute as a single sharp peak during stable CAPD. A single sTNFRI peak was observed both during peritonitis and stable dialysis, corresponding with a molecular weight of ~69 kD. However, for sTNFRII multiple peaks were observed during stable CAPD (~69 and 106 kD), on the first peritonitis day (~45, 69 and 106 kD) and on the second peritonitis day (~55 and 106 kD).

Discussion

Immunoreactive TNF-α could be detected in nearly all dialysates after an overnight dwell, but only occasionally and in lower concentrations during and after a standard four-hour peritoneal permeability test. In case of peritoneal infection a median 16-fold increment occurred for TNF-α on the first day, whereas three- to fourfold increases of sTNFRs were found on the second day of peritonitis, significantly later than TNF-α. Soluble TNF-receptors markedly exceeded TNF-α under all circumstances, both in dialysate and in the circulation.

Serum concentrations of all three agents were considerably higher than those described for healthy individuals. Similar elevated values have been found in uremic patients before or during treatment with hemo- or peritoneal dialysis by other groups [18–21, 28, 29]. These high concentrations are probably related to the presence of chronic renal failure, which leads to either a decreased clearance or an increased release of these substances. Indeed, an inverse relationship has been described between serum soluble TNF-receptors and renal function [19, 30]. Some caution, however, is warranted concerning the interpretation of increased TNF-α levels, since TNF-α binding proteins—like soluble receptors—can interfere with the measurement of TNF-α in biological fluids. For example, Haran et al could no longer detect differences in serum TNF-α concentrations between hemodialysis patients and healthy controls, when a modified assay was used [31]. The TNF-α assay used in the present study detects TNF-α both in its free and receptor-bound form. As will be discussed below, our data indicate TNF-α was present in dialysate mainly in a free form.

Peritoneal infection did not affect serum concentrations of TNF-α, sTNFRI or sTNFRII. This has been reported for TNF-α before [32] and underlines that CAPD-related peritonitis reflects a local inflammatory process. In contrast to serum, dialysate concentrations of TNF-α and sTNFRs were increased in the acute inflammatory phase. It is not unexpected that peak dialysate concentrations of sTNFRs occurred after the TNF-α peak. TNF-α is a proximal mediator, able to induce release of other substances, including its own receptors [33]. A similar time course has been shown for serum concentrations of human volunteers with experimental endotoxemia [34]. These *in vivo* studies indicate that TNF-α probably regulates its own activity.

As might be expected, given the excess of soluble TNF-receptors, bioactivity of peritoneal TNF-α could only be detected when TNF-α was present in very high concentrations during peritonitis. This seems contradictory to another study [32], that

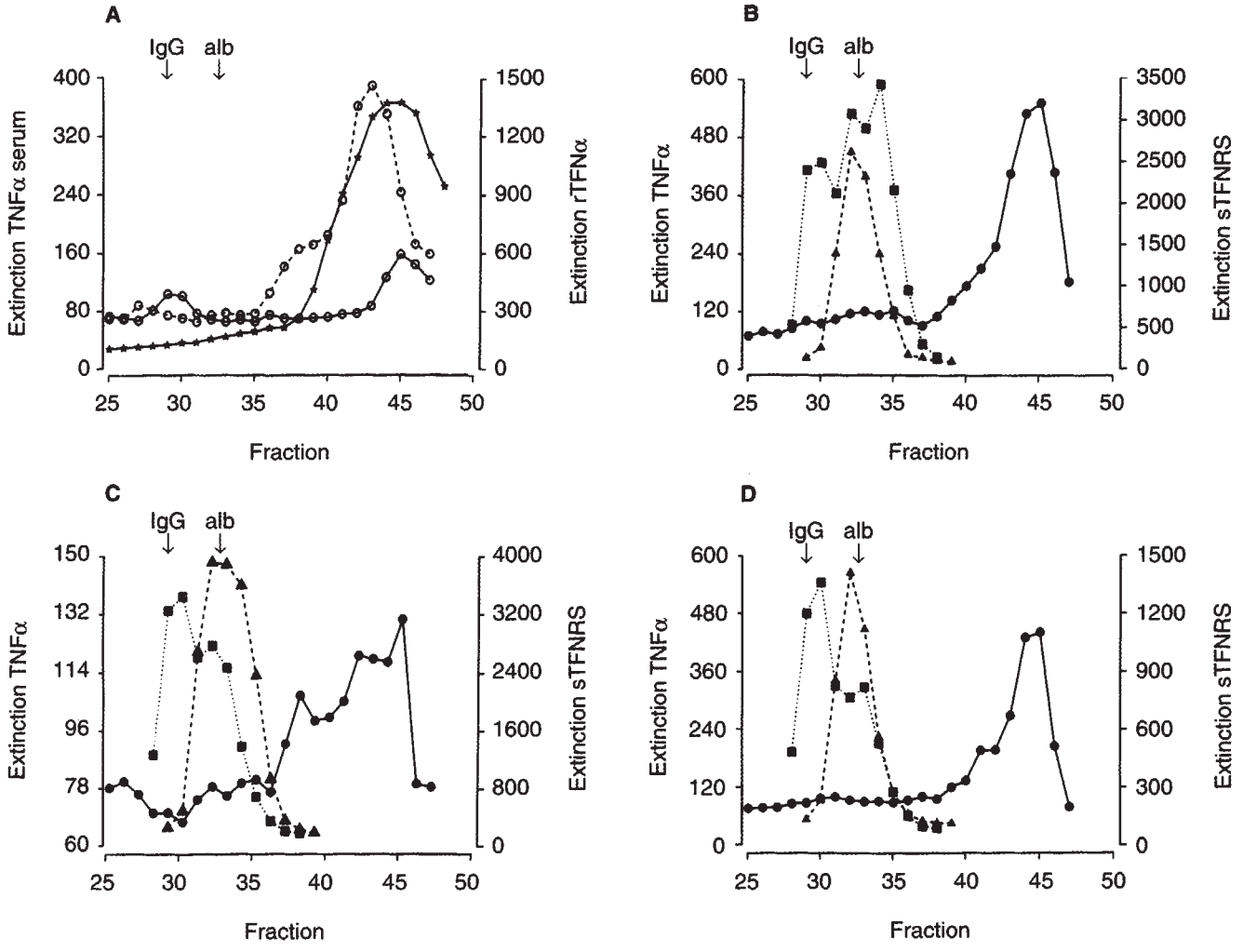

Fig. 6. *Representative patterns of gel chromatography of TNF-α, sTNFRI and sTNFRII performed in different situations.* IgG (150 kD) and albumin (67 kD) were used as molecular weight standards. **A.** Elution profile of the applied control samples for TNF-α: * recombinant monomeric TNF-α (17 kD); —⊖— serum from septic patient with normal kidney function; —⊖— serum from septic patient with acute renal failure. **B.** Elution profile during the first peritonitis day. **C.** Elution profile of dialysate from stable CAPD patient. **D.** Elution profile on the second peritonitis day. Symbols are: (●) TNF-α; (▲) sTNFRI; (■) sTNFRII.

reported bioactive TNF-α both in stable and infectious circumstances. The discrepancy is possibly due to the use of different cell lines to determine bioactivity. In addition, these authors reported similar peritoneal TNF-α concentrations during stable CAPD and peritonitis. However, they compared long, uninfected night dwells to shorter peritonitis dwells. Since more TNF-α accumulates intraperitoneally, the longer the dwell time, increased TNF-α concentrations during infection might have remained unnoticed.

In case of stable CAPD transperitoneal transport of TNF-α and sTNFRs was indicated, rather than intraperitoneal release. First, D/S ratios were higher, the lower the molecular weight of the substance, consistent with size selective transport from blood to dialysate [27]. Secondly, their D/S ratios were in the range between those of marker proteins for peritoneal transport with a molecular weight approaching that of TNF-α, sTNFRI or sTNFRII. Thirdly, the strongest correlations were found for D/S ratios between TNF-α and β_2-microglobulin (11.8 kD), sTNFRI and albumin (69 kD) and sTNFRII and IgG (150 kD). Fourthly, the D/S ratios of these mediators were unrelated to that of IL-6, a locally produced 26 kD cytokine. Finally, the observed *in vivo* D/S ratios of TNF-α, sTNFRI and sTNFRII were consistent with predicted values for transperitoneal transport of 17 kD, 55 kD and 75 kD solutes, respectively. Our data on TNF-α are in agreement with those from Hain et al [11], who also suggested transperitoneal transport, but contradict data from Floege et al [12] and Mariano et al [32]. However, they did not compare D/S ratios of TNF-α to those of marker proteins for transport and locally released solutes.

In case of acute peritonitis significantly higher D/S ratios than theoretically expected for their molecular sizes were observed for TNF-α and sTNFRII. This can be explained either by intraperitoneal release or by the presence of smaller fragments, that will be transported at a higher rate than the full size solutes. Gel permeation chromatography was therefore performed on dialysate to distinguish between these possibilities. TNF-α always eluted in the same fraction as recombinant monomeric TNF-α,

irrespective whether infection was present or not. It implies that TNF-α was constantly present in a monomeric 17 kD form. Interestingly, both soluble receptors eluted in fractions distinct from TNF-α. These data therefore indicate intraperitoneal release of free, monomeric 17 kD TNF-α during acute peritonitis, unbound to soluble receptors. This would be consistent with our inability to detect bioactive TNF-α in most dialysate samples; only trimeric TNF-α is considered to be biologically active [35]. In dialysate obtained during stable CAPD did TNF-α not elute as a single sharp peak. This was potentially caused by the tenfold concentration step that was necessary to obtain sufficient TNF-α from dialysate samples of stable patients to perform chromatography. Alternatively, low concentrations of dimeric or trimeric TNF-α might have been present. In contrast to TNF-α, the existence of smaller fragments of sTNFRII in peritoneal fluid could not be excluded. Thus, in addition to local sTNFRII release during acute peritonitis, diffusion of smaller fragments, that were transported at a higher rate than the entire 75 kD receptor, could have occurred.

The low dialysate TNF-α concentrations during stable CAPD, which were biologically inactive, do not suggest unfavorable effects on the maintenance of peritoneal membrane function in terms of fibrosis or angiogenesis. These dialysate concentrations do not indicate that the instillation of commercially available dialysis solutions leads to local release of TNF-α. It implies that dialysate TNF-α concentrations are not useful for the establishment of improved biocompatibility when new dialysis fluids are tested. In case the instillation of a new fluid would lead to a marked rise in TNF-α concentrations, such a fluid would be very bioincompatible. Although TNF-α was produced locally during peritonitis, this occurred only in the acute phase; peritoneal concentrations became normal within one week. Furthermore, soluble TNF-receptors increased as well, probably to regulate TNF-α activity. In case of infection synthesis of TNF-α very likely reflects a physiological response to infectious agents. Endogenous TNF-α is, for example, required *in vivo* in mice for survival of otherwise lethal peritonitis [36]. Peritoneal macrophages, which form the first line of defense within the peritoneal cavity, are a likely source of TNF-α [37]. These cells are involved in a cytokine network with mesothelial cells, which line the entire cavity and are capable of releasing inflammatory mediators like IL-6, IL-8 and eicosanoids [38–40], and possibly also with fibroblasts and neutrophils [41]. It is not currently known which of the resident peritoneal cells express soluble TNF-receptors. Nevertheless, it is tempting to speculate that upon bacterial challenge macrophages release the proinflammatory cytokine TNF-α. TNF-α might subsequently activate other peritoneal cells for additional TNF-α release and shedding of sTNFRII.

It is concluded that TNF-α and its soluble TNF-receptors appear in dialysate of stable CAPD patients by transperitoneal transport from the circulation. Local intraperitoneal release probably occurs for TNF-α and sTNFRII only in case of acute peritoneal infection.

Acknowledgments

This study was partly supported by the Baxter Extramural Grant Program. The authors thank professor L. Arisz for critically reviewing the manuscript. Drs. R.E. Reddingius and A.M. Koster are acknowledged for their statistical advice, especially regarding their development of the modified *t*-test used to distinguish between transperitoneal and local release. Drs. C.A. Visser and J.J.E. Brouwer-Steenbergen are acknowledged for performing the bioassay. The nursing staff of the CAPD unit is acknowledged for technical help, J.K. Hiralall for analytical help and M. Zeeman for secretarial assistance.

Reprint requests to Dr. Désirée Zemel, Renal Unit, Department of Medicine, Academic Medical Center, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands.

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